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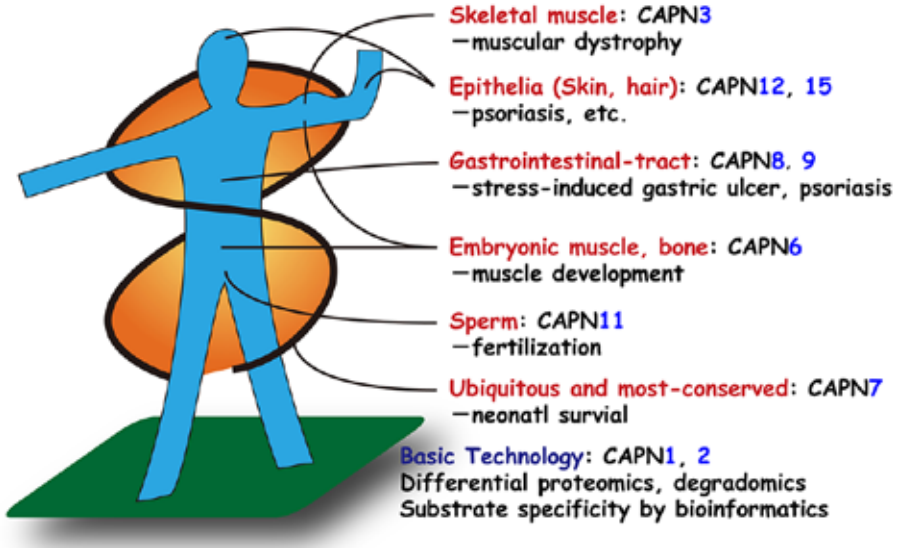
Calpain

Project Leader **Yasuko Ono** Calpain Project

Calpain: Structure-Function Relationships

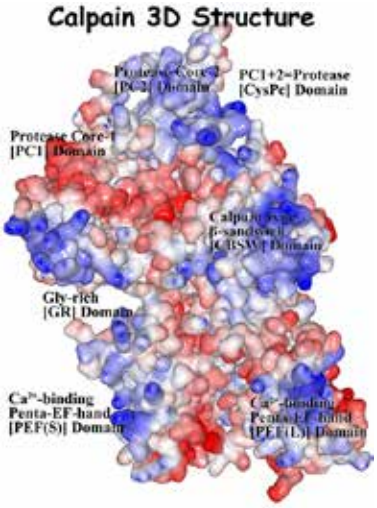
Exploring calpain-mediated biological modulation

Proteins are chains of amino acids, and their functions change when they are cut or partially cut. Calpains are enzymes that perform such "cuts" or "limited proteolytic processing" in cooperation with calcium. Humans have 15 calpain species. Defects of these species cause various deficiencies, such as muscular dystrophy, stomach ulcer, and embryonic lethality.



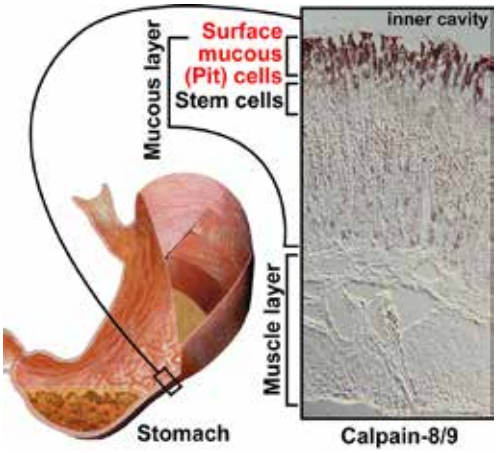
"Translational research involving calpains is still at the developmental stage. We need to learn more about the calpains themselves, as well as their impact on various physiological systems and molecular pathways." (Nat.Rev.Drug Discov.2016).

In this project, we aim to understand the biology of calpains, and translate this knowledge into improvements in health.

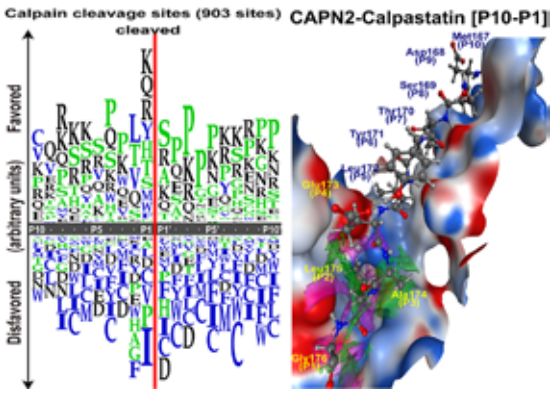


Calpains in health and disease

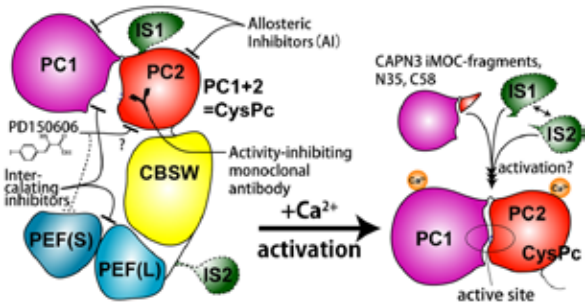
Some calpains, predominantly these expressed in specific tissue(s), are associated with genetic diseases; *e.g.*, defects in CAPN3 cause muscular dystrophy. Other calpains with more ubiquitous expression cause lethality if deficient. In addition, some calpain species express their activity through unique and unexpected mechanisms, such as intermolecular complementation (CAPN3), heterodimerization (CAPN8/9), etc.
To explore how calpains protect our health, analyses of cells/mice lacking function of specific calpain species or expected targets are being performed. We are also improving research platforms for studying calpains using proteomics, genetics, and bioinformatics.



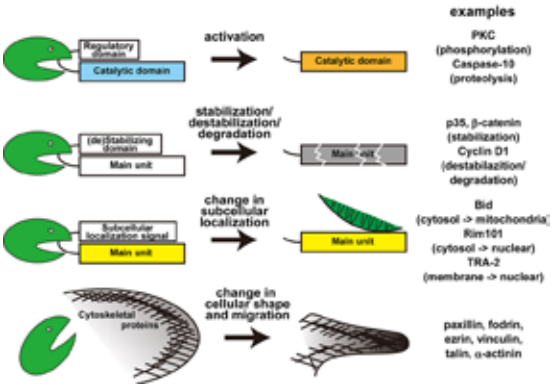
Protection of epithelial cells by heterodimeric calpain, G-calpain



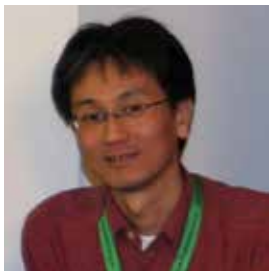
Characterization of calpain-substrate interface



Strategy for activity regulation of CAPN3



Multiplicity of calpain actions



Shoji Hata, Ph.D.
Calpains in epithelial function and tissue development

Fumiko Shinkai-Ouchi, Ph.D.
Proteomic analysis of muscular dystrophy and calpain substrate specificities

Aya Noguchi, Ph.D.
Cross talk of calpain and other proteolytic systems

Calpain

Project Leader **Noriyuki Matsuda** Ubiquitin Project

Ubiquitin-Mediated Mitochondrial Quality Control: A shield against Parkinson's disease

Ubiquitin is well-known as a signal for proteasome-dependent degradation; however, it also functions in autophagic degradation. Increasing evidence indicates that selective autophagy functions in intracellular quality control by using ubiquitin tags to delineate aggregated proteins and damaged organelles for degradation.

In 2000, Dr. Mizuno and Dr. Hattori (Juntendo Univ.), in collaboration with Dr. Suzuki and Dr. Tanaka (TMIMS) reported for the first time that Parkin, which is a causative gene product for familial Parkinson's disease (PD), is a ubiquitin-protein ligase (Nat. Genet. 2000). In addition, the identification of another gene PINK1 that is linked to familial forms of PD (Science 2004) has revealed that phosphorylation, ubiquitylation, and mitochondrial integrity are key factors in disease pathogenesis. Nevertheless, the exact mechanism underlying the functional interplay between Parkin and PINK1 remained an enigma. We are investigating how PINK1 and Parkin cooperate to keep mitochondrial integrity against mitochondrial stresses.

"We found that low-quality mitochondria are marked with ubiquitin for selective degradation, and the key factors in this process are PINK1 (a mitochondrial kinase) and Parkin (a ubiquitin ligase), two proteins implicated in Parkinson's disease."

PINK1 is a mitochondrial Ser/Thr kinase, whereas Parkin is a ubiquitin-protein ligase that catalyzes ubiquitylation of diverse mitochondrial outer membranous proteins (J. Cell Biol. 2010). We revealed that PINK1 is rapidly and constitutively degraded under steady-state conditions in a mitochondrial membrane potential-dependent manner, but that a loss in mitochondrial membrane potential stabilizes PINK1 mitochondrial accumulation (J. Cell Biol. 2010). Previously our group and others found that PINK1 acts as an upstream factor for Parkin, but how PINK1 activates latent Parkin and recruits cytoplasmic Parkin to damaged mitochondria was still obscure.

Yamano K, Wang C, Sarraf S, Münch C, Kikuchi R, Noda N, Hizukuri Y, Kanemaki M, Harper W, Tanaka K, Matsuda N, and Youle R. (2018) "Endosomal Rab cycles regulate Parkin-mediated mitophagy." ***eLife*** 7: e31326

Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, and Matsuda N. (2015) "Phosphorylated ubiquitin chain is the genuine Parkin receptor." ***J. Cell Biology*** 209:111-128

Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T, Endo T, Fon E-A, Trempe J-F, Saeki Y, Tanaka K, and Matsuda N. (2014) "Ubiquitin is phosphorylated by PINK1 to activate Parkin." ***Nature*** 510, 162-166.

Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, Tani N, Kimura M, Go E, Koyano F, Funayama M, Shiba-Fukushima K, Sato S, Shimizu H, Fukunaga Y, Taniguchi H, Komatsu M, Hattori N, Mihara K, Tanaka K, and Matsuda N. (2012) "PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria." ***Nature Commun.*** 3 : e1016 (10 pages).

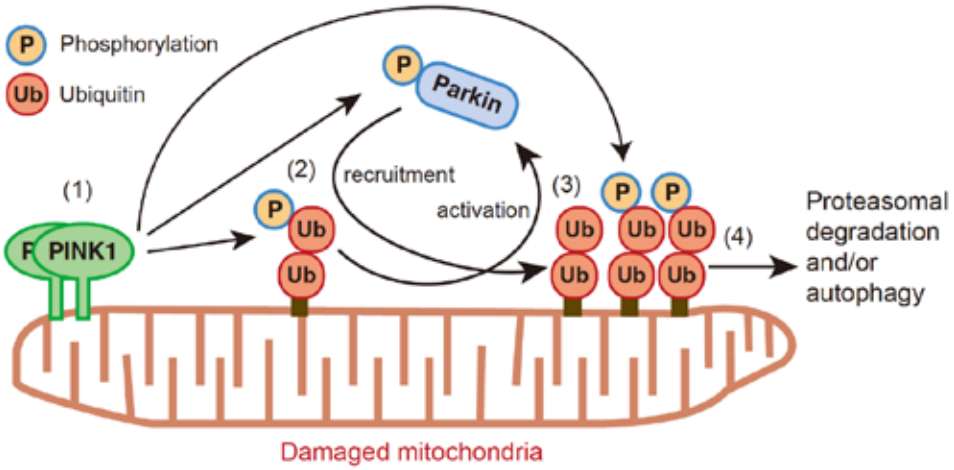
Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier C, Sou Y-S, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, and Tanaka K. (2010) "PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy." ***J. Cell Biology*** 189: 211-221.

Ubiquitin

We have found that PINK1 phosphorylates both Parkin and ubiquitin, and both types of phosphorylation contribute to activation of Parkin E3, with Ser65 phosphorylated ubiquitin acting as a Parkin activator (Nature 2014). In addition, we found that phosphorylated ubiquitin chains function as genuine Parkin receptors, recruiting it to depolarized mitochondria (J. Cell Biol. 2015). Thus ubiquitin phosphorylation allows us to comprehensively understand how PINK1 regulates Parkin to prevent Parkinson's disease.

Our study has revealed that PINK1 and Parkin cooperate in the recognition, labeling, and clearance of damaged (i.e., depolarized) mitochondria by selective mitochondrial autophagy (mitophagy). To date, ubiquitylation has been a well-known post-translational modification; however, it is becoming increasingly clear that modification of ubiquitin itself plays a critical cellular function as demonstrated by the role of S65-phosphorylated ubiquitin functions in mitochondrial quality control.

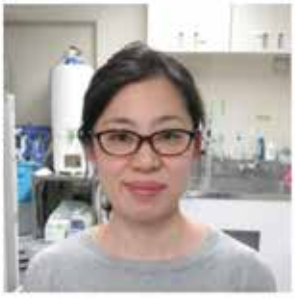
"We believe that a big mystery in mitochondrial quality control has been unraveled, and our work has established new principles of how a simple ubiquitin tag plays more varied roles than expected."



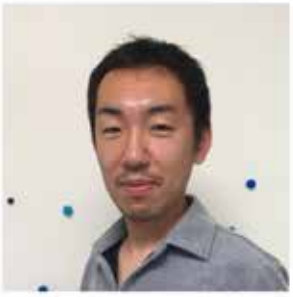
Our model for PINK1- and Parkin-catalyzed ubiquitylation for mitochondrial quality control.
We have revealed that accumulated PINK1 on damaged mitochondria (1) phosphorylates Parkin and ubiquitin, which (2) induces Parkin activation and its recruitment to the phosphorylated ubiquitin chain. Activated Parkin produces more ubiquitin chain (3), and the resultant ubiquitin is phosphorylated by PINK1 in a feed forward cycle. Parkin thus functions as an amplifier of the ubiquitin chain on depolarized mitochondria (4) for degradation.

Members

Yukiko Yoshida
Organellophagy via glycoprotein-specific ubiquitin ligase



Fumika Koyano
Molecular mechanism underlying Parkin-catalyzed ubiquitylation



Koji Yamano
Membrane dynamics upon mitochondrial quality control

Ubiquitin

Project Leader **Takahiko Hara** Stem Cell Project

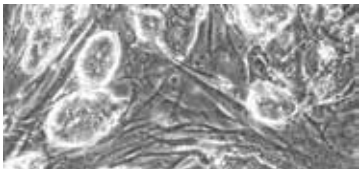
Blood regeneration from ESC/iPSC and development of novel anti-cancer drugs

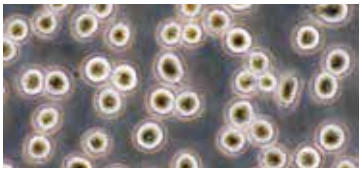
Dr. Yamanaka's inducible pluripotent stem cell (iPSC) technology has opened a new avenue to overcome incurable diseases by cell transplantation. In 2011, we discovered that overexpression of Lhx2 in hemogenic mesodermal cells resulted in *ex vivo* expansion of transplantable hematopoietic stem cells (HSCs) from mouse embryonic stem cells (ESCs) and iPSCs. Since then, we have been improving this system and applying this method to human iPSCs. We believe that comparison of the *in vitro* differentiation capacity of hematopoietic cells between mouse and human iPSCs will uncover novel and fundamental aspects of human HSC development.

"We are making efforts to derive HSCs from human iPSCs in vitro. We are also developing novel anti-leukemia drugs and chemokine-based anti-cancer drugs."

The presence of cancer stem cells has been proposed in various types of human cancer. As with tissue stem cells, cancer stem cells reside in a niche and stay dormant, thereby surviving chemotherapy and radiotherapy. Presumably, both tissue and cancer stem cells commonly express critical transcriptional regulators and signal transducers. We have already identified DDX1 and PTPN23 as essential molecules for the onset of testicular tumors.

In 2007, we discovered that CXCL14, a CXC-type chemokine, is one of the causative factors for obesity-associated diabetes. In contrast, CXCL14 is known to possess tumor-suppressive activity against lung and oral carcinomas. Recently, we discovered that CXCL14 binds to CXCR4 with high affinity, thereby inhibiting the CXCL12-mediated cell migration. This could be one of the underlying mechanisms of the CXCL14's anti-tumor function. We are vigorously investigating physiological roles of CXCL14 and its action mechanisms. CXCL14 is a promising tool for developing novel anti-cancer and anti-diabetes drugs.





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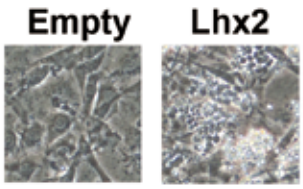
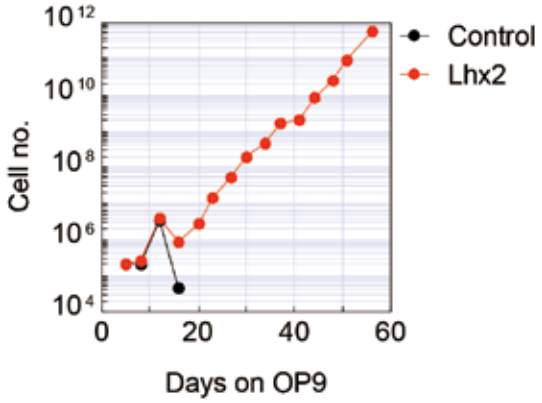
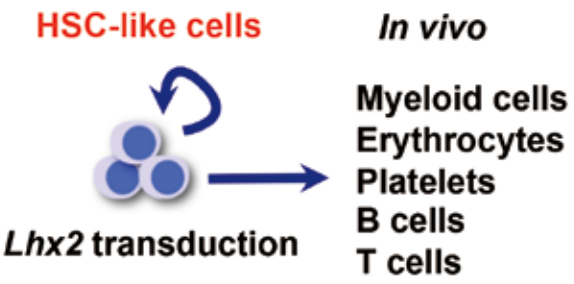
Stem Cell



Topic 1

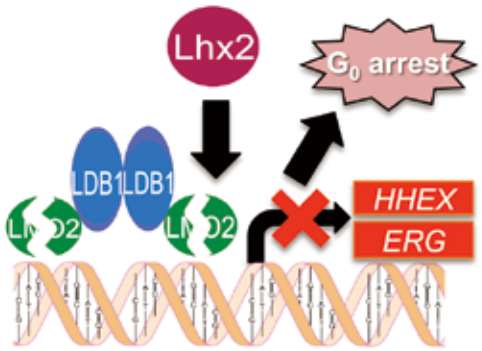
Enforced expression of *Lhx2* leads to robust *ex vivo* production of HSC-like cells from mouse ESCs/iPSCs.





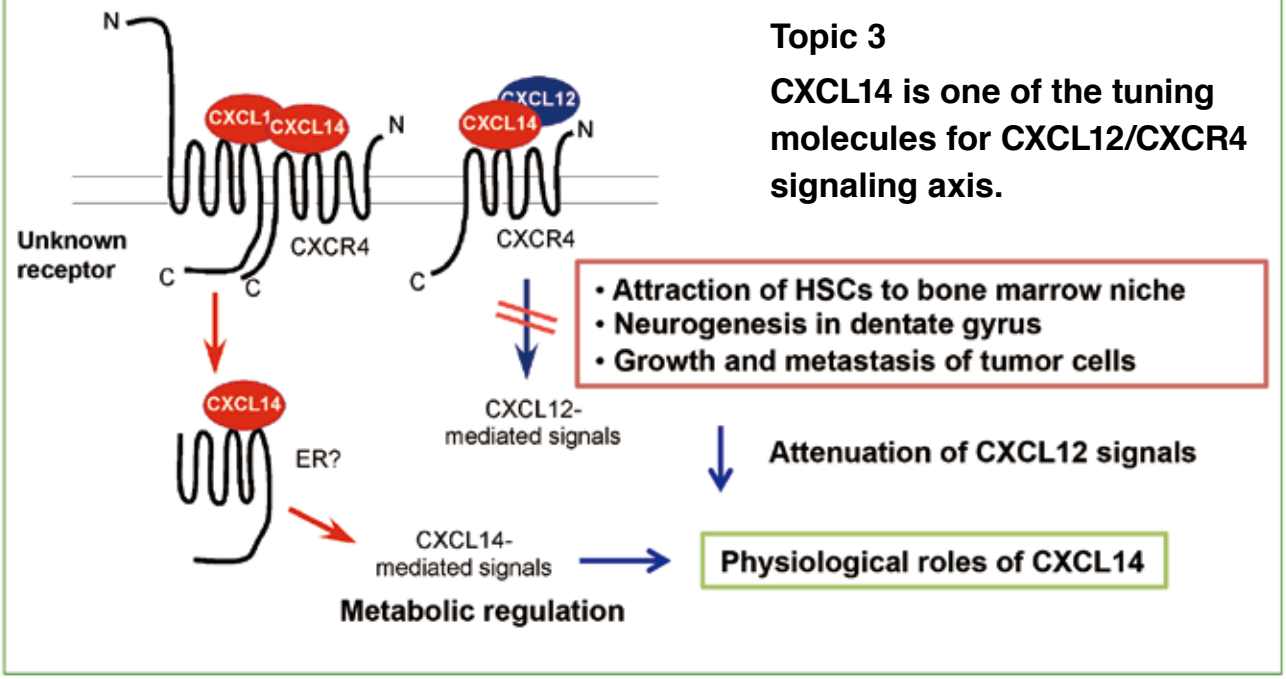
Topic 2

Lhx2 induces degradation of Lmo2 protein, thereby inhibiting proliferation of human T-cell acute lymphoblastic leukemia (T-ALL) cells.

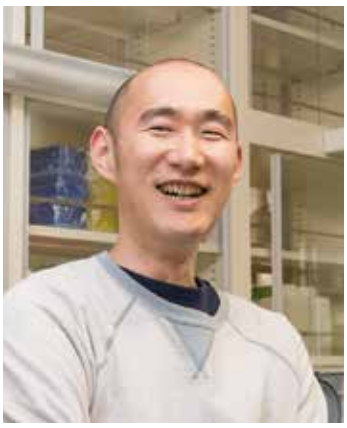


Topic 3

CXCL14 is one of the tuning molecules for CXCL12/CXCR4 signaling axis.



Stem Cell



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Miyaoka Y, Mayerl SJ, Chan AH, and Conklin BR. (2018) "Detection and Quantification of HDR and NHEJ Induced by Genome Editing at Endogenous Gene Loci Using Droplet Digital PCR." ***Methods Mol. Biol.*** 1768: 349-362.

Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG, Elefanty AG, Miyaoka Y, Mandegar MA, Conklin BR, Neunlist M, Brugmann SA, Helmrath MA, and Wells JM. (2017) "Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system." ***Nat. Med.*** 23: 49-59.

Miyaoka Y, Chan AH, and Conklin BR. (2016) "Using Digital Polymerase Chain Reaction to Detect Single-Nucleotide Substitutions Induced by Genome Editing." ***Cold Spring Harb. Protoc.*** 2016:688-692.

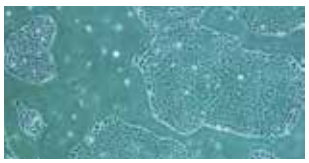
Miyaoka Y, Berman JR, Cooper SB, Mayerl SJ, Chan AH, Zhang B, Karlin-Neumann GA, and Conklin BR. (2016) "Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing." ***Sci. Rep.*** 6: 23549.

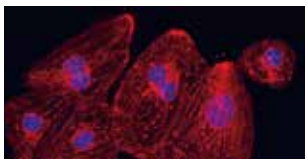
Miyaoka Y, Chan AH, Judge LM, Yoo J, Huang M, Nguyen TD, Lizarraga PP, So PL, and Conklin BR. (2014) "Isolation of single-base genome-edited human iPS cells without antibiotic selection." ***Nat. Methods*** 11: 291-293.

Project Leader **Yuichiro Miyaoka** Regenerative Medicine Project

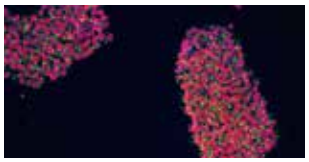
Genome Editing in Human iPS Cells: To study and cure genetic disorders

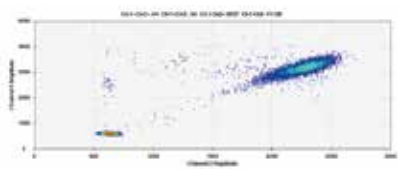
Genome editing technology allows us to rewrite the genetic information in virtually any species and any cell type including human cells. To study the pathogenesis of human diseases at the molecular level, and to develop new therapies using genome editing, we need appropriate human cellular models. Our focus is on human iPS (induced pluripotent stem) cells, a type of pluripotent stem cell that can be generated from patients' cells by introduction of specific transcription factors, and differentiated into other cell types. Our goal is to use genome editing of iPS cells to both model human diseases, and develop new therapies.





"Our goal is to develop methods to precisely and efficiently edit the genome in human iPS cells to allow us to develop both disease models using human cells, and new therapies for these diseases."



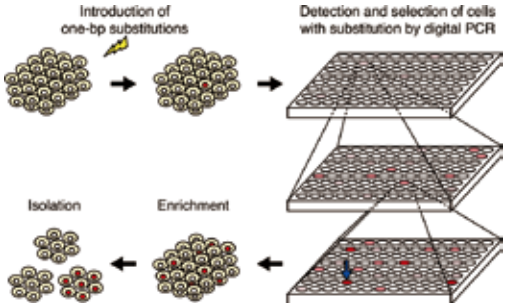


We developed an efficient method to isolate iPS cell lines containing a single nucleotide substitution. The nucleotide substitution is created by genome editing based on digital PCR, and isolation is accomplished by repeated limited dilutions in the absence of selection markers (Miyaoka, Nat. Methods 2014). Using this method, we are analyzing the pathogenesis of cardiomyopathy caused by point mutations of RBM20 (RNA-binding motif protein 20) in isogenic cardiomyocytes derived from genome-edited iPS cells. We are also improving the precision and efficiency of genome editing technology, and developing new therapies based on correcting mutations in iPS cells from patients. In addition, we are developing a strategy to directly edit the genome in cells in the human body.

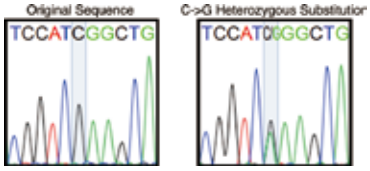
Regenerative Medicine

Changing a Single DNA Base-pair out of Thirty Billion

Single point mutations are often responsible for genetic disorders. Thus, the development of techniques to generate single point mutations is important for both modeling and curing diseases. However, thus far, it had been difficult to make specific single base-pair (bp) substitutions in the 3 billion-bp human genome. We have developed a method for isolating iPS cells with single-bp substitutions by combining genome editing, and serial limited dilutions using digital PCR.



Isolation of iPS cells with single nucleotide substitution

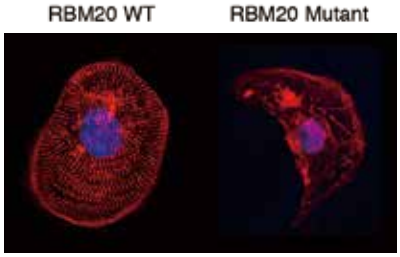


Isolated iPS cells with C→G substitution

Using this method, we can efficiently introduce single-bp substitutions at any location in the genome, allowing us to develop iPS cell-based disease models and transplantation therapies.

Heart Failure in a Dish

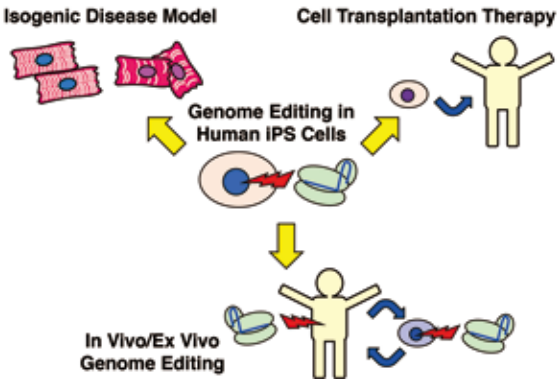
By editing the genome of iPS cells, we can study pathogenic mechanisms of genetic disorders in any cell type in a dish. For example, a point mutation in RBM20(a cardiomyopathy mutation) introduced into iPS cells caused abnormal sarcomere structures (a functional unit of muscle contraction visualized as red stripes), when these cells were differentiated into cardiomyocytes. These cells can serve as a platform for drug screening.



Sarcomere (red) and nucleus (blue) in iPS cell-derived cardiomyocytes

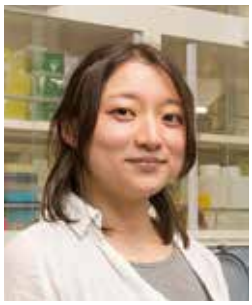
Development of Precise Ways to Edit the Genome

Current genome editing tools including CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) have revolutionized our ability to modify the genetic information in cells. However, these tools still need to be improved for accuracy and efficiency when used in therapies. Therefore, we are developing a more precise and efficient way to edit the genome by modifying the Cas9 nuclease, and the guide RNA that directs Cas9 to the target regions. These improvements are necessary for further development of genome editing-based therapies.

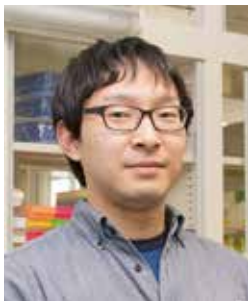


Genome editing in iPS cells to study and cure disease

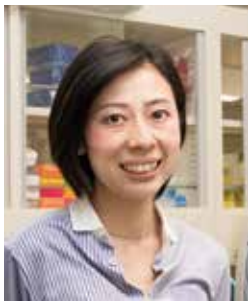
Members



Tomoko Kato-Inui



Gou Takahashi

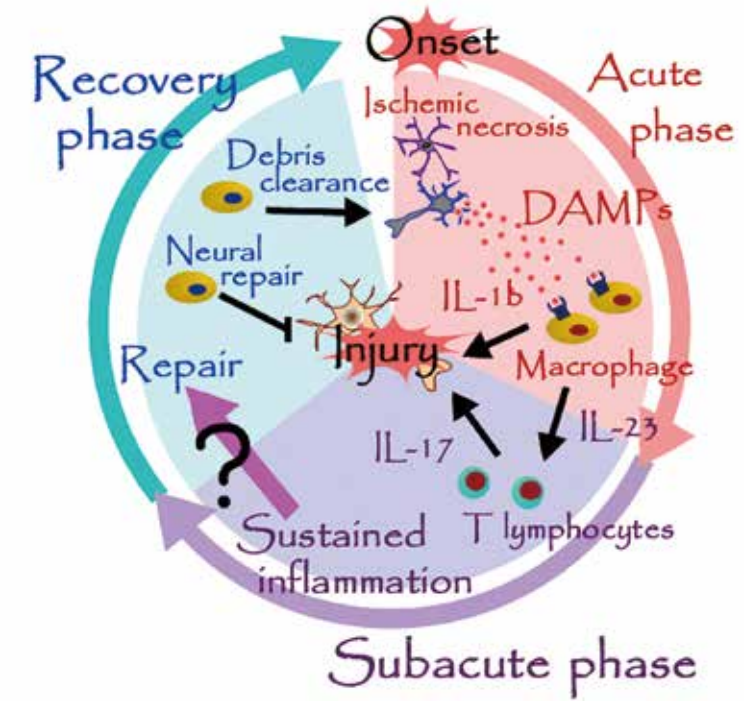


Szuyin Hsu

Regenerative Medicine

Project Leader **Takashi Shichita** Stroke Renaissance Project

Sterile Inflammation After Ischemic Stroke



"What triggers neural repair after stroke?"

We have identified peroxiredoxin family proteins as DAMPs (damage associated molecular patterns) which trigger post-ischemic inflammation (***Nat. Med.*** 2012). DAMPs induce IL-23 production from infiltrating macrophages and neutrophils, and this sustains the inflammation after ischemic stroke by promoting IL-17 production of gdT lymphocytes (***Nat. Med.***2009). Cerebral post-ischemic inflammation resolves several days after the stroke onset. The clearance of DAMPs from ischemic brain through MSR1, a scavenger receptor, plays a pivotal role in the resolution of sterile inflammation after ischemic stroke (***Nat. Med.*** 2017). Now our question is how cerebral post-ischemic inflammation switches into the process of neural repair.

Tsuyama J, Nakamura A, Ooboshi H, Yoshimura A, and Shichita T. (2018) "Pivotal role of innate myeloid cells in cerebral post-ischemic sterile inflammation." ***Semin. Immunopathol.***

Shichita T, Ito M, Morita R, Komai K, Noguchi Y, Ooboshi H, Koshida R, Takahashi S, Kodama T, and Yoshimura A. (2017) "Mafb prevents excess inflammation after ischemic stroke by accelerating clearance of danger signals through MSR1." ***Nat. Med.*** 23(6): 723-732.

Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, Takada I, Sekiya T, Ooboshi H, Kitazono T, Yanagawa T, Ishii T, Takahashi H, Mori S, Nishibori M, Kuroda K, Akira S, Miyake K, and Yoshimura A. (2012) "Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain." ***Nat. Med.*** 18(6): 911-917.

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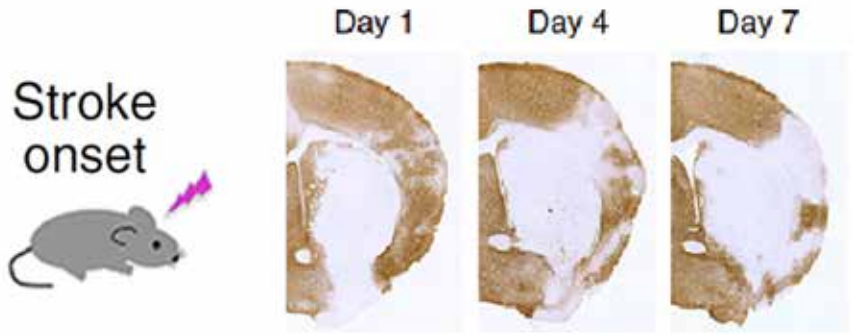
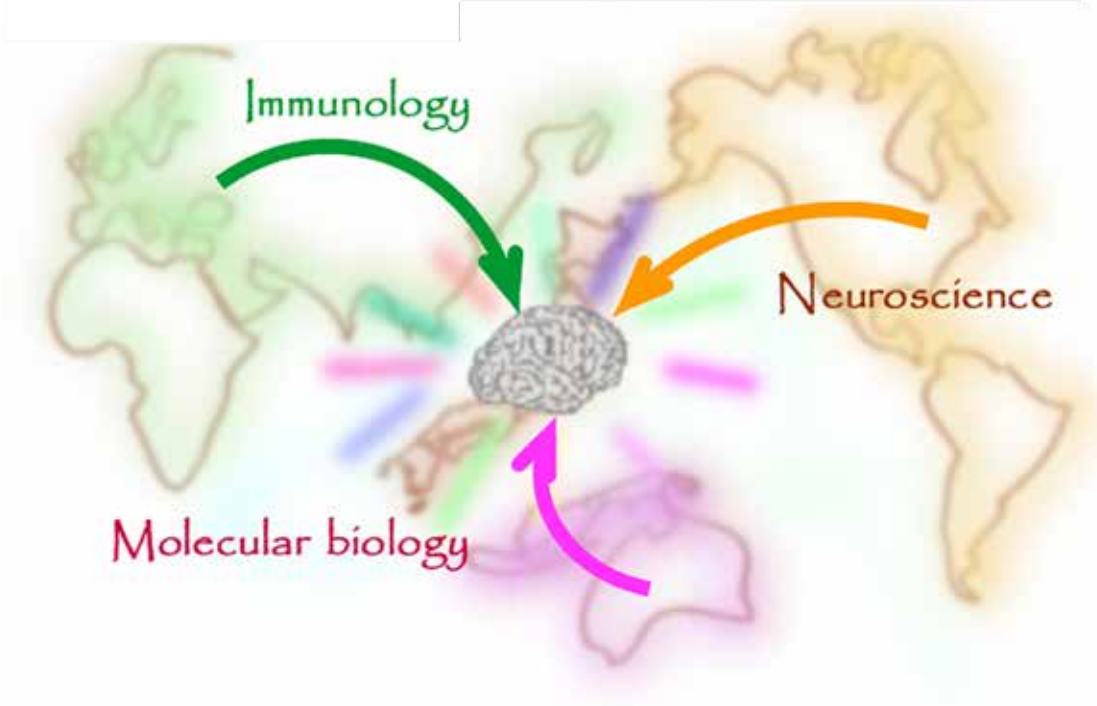
Stroke Renaissance

Stroke Renaissance Project

Stroke is a common cause of cause of severe disability and death worldwide; however, few therapeutic agents have been shown to improve the neurological deficits of stroke patients.



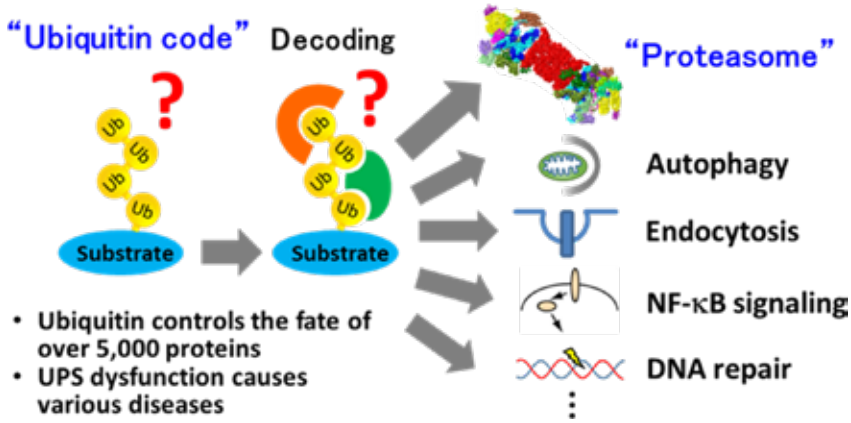
In the project, we are studying the detailed molecular mechanisms underlying the recovery of the brain after stroke. New research methods and techniques which have been recently developed in the field of immunology or neuroscience are allowing us to investigate the precise process of inflammation and regeneration in the injured brain after stroke. The purpose of our project is to develop a new therapeutic method for promoting the recovery of neurological function in patients with cerebrovascular diseases.



Stroke Renaissance

Laboratory Head **Yasushi Saeki** Protein Metabolism Laboratory

The Ubiquitin Proteasome System: Elucidation of Fundamental and Pathophysiological Mechanisms



The ubiquitin-proteasome system (UPS) plays a pivotal role in proteostasis and controls almost all cellular functions by selective protein degradation. As the maintenance of protein homeostasis is essential to human health, dysfunction of the UPS due to stresses, age-associated changes, or gene mutations causes various diseases such as cancers, inflammation, and neurodegeneration. However, we do not yet know the overall principles behind ubiquitin signaling, decoding mechanisms, and the proteasome. We aim to elucidate the fundamental mechanisms of the ubiquitin code as well as proteasome function and integrate this information into pathophysiology, to develop therapeutic strategies for UPS-related diseases.

Research Projects

1. Proteasome Dynamics and Pathophysiology

The proteasome is a highly organized proteolytic machine that degrades ubiquitylated proteins in an ATP-dependent manner. We have characterized the structure, assembly pathway, and substrate targeting mechanism of the proteasome. We found that the proteasome dynamically changes its intracellular localization and its accessory proteins under various stresses to restore proteostasis. Currently, we are generating knock-in mice to visualize proteasome localization and activity to analyze physiological changes of the proteasome during stress and aging. Furthermore, we have generated model mice with proteasomal gene mutations derived from patients with neurodevelopmental disorders. Using these mutant mice, we will elucidate the pathophysiology of proteasome mutations at the whole-body level.

Tsuchiya H, Burana D, Ohtake F, Arai N, Kaiho A, Komada M, Tanaka K, and Saeki Y. (2018) "Ub-ProT reveals global length and composition of protein ubiquitylation in cells." ***Nature Commun.*** 9, 524.

Ohtake F, Tsuchiya H, Saeki Y, and Tanaka K. (2018) "K63 ubiquitylation triggers proteasomal degradation by seeding branched chains." ***Proc. Natl. Acad. Sci. USA.*** 115, E1401-E1408.

Xie X, Matsumoto S, Endo A, Fukushima T, Kawahara H, Saeki Y, and Komada M. (2018) "Deubiquitylases USP5 and USP13 are recruited to and regulate heat-induced stress granules through deubiquitylating activities." ***J. Cell. Sci.*** 131, jcs201856

Tsuchiya H, Ohtake F, Arai N, Kaiho A, Yasuda S, Tanaka K, and Saeki Y. (2017) "In vivo ubiquitin linkage-type analysis reveals that the Cdc48-Rad23/Dsk2 axis contributes to K48-linked chain specificity of the proteasome." ***Mol. Cell*** 66, 485-502.

Ohtake F, Saeki Y, Ishido S, Kanno J, and Tanaka K. (2016) "The K48-K63 branched ubiquitin chain regulates NF-κB signaling." ***Mol. Cell*** 64, 251-266.

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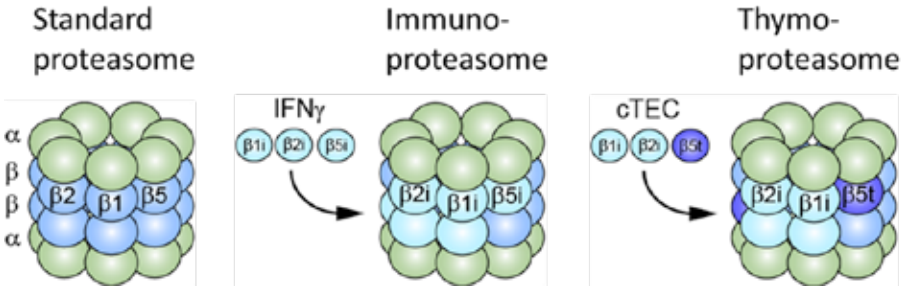
Pack Chan-Gi, Yukii H, Toh-e A, Kudo T, Tsuchiya H, Kaiho A, Sakata E, Murata S, Yokosawa H, Sako Y, Baumeister W, Tanaka K, and Saeki Y. (2014) "Quantitative live-cell imaging reveals molecular dynamics and cytoplasmic assembly of the 26S proteasome." ***Nature Commun.*** 5, 4396

Saeki Y, Toh-e A, Kudo T, Kawamura H, and Tanaka K. (2009) "Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle." ***Cell*** 137, 900-913.

Protein Metabolism

2. Roles of Specialized Proteasomes in Cell-Mediated Immunity

The proteasome has acquired diversity in the catalytic β subunits, which likely evolved during the acquisition of adaptive immunity. To date, we have discovered the vertebrate-specific alternative proteasomes, which we named the "immunoproteasome" and the “thymoproteasome”. Whereas the immunoproteasome plays a specialized role as a professional antigen-processing enzyme in cell-mediated immunity, the thymoproteasome is involved in the development of CD8+T cells in the thymus; i.e., it has a key role in the generation of the MHC class I-restricted CD8+T cell repertoire during thymic selection called “positive selection”. Currently, we are conducting a deep proteomic screen to validate the positive selection model.



Keiji Tanaka
(The chairperson of TMIMS)

Murata S, Takahama Y, Kasahara M, and Tanaka K. (2018) “The immunoproteasome and thymoproteasome: functions, evolution and human disease.” ***Nature Immunol.*** 19, 923-931.

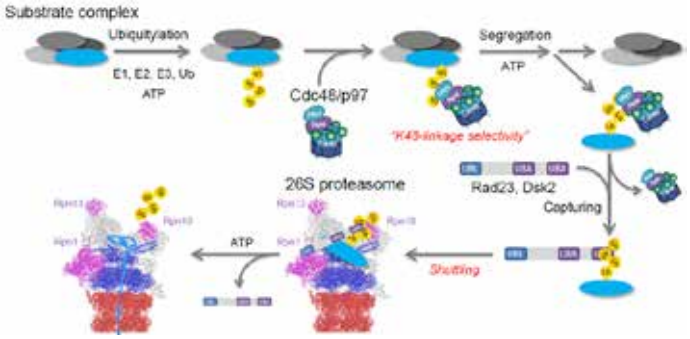
Murata S, Sasaki K, Kishimoto T, Niwa S, Hayashi H, Takahama Y, and Tanaka K. (2007) “Regulation of CD8+ T cell development by thymus-specific proteasomes.” ***Science*** 316, 1349-1353.

3. Deciphering the Ubiquitin Code

Different polyubiquitin chain linkages direct substrates to distinct pathways. This is known as the ‘ubiquitin code’. We have developed a highly sensitive MS/MS-based quantification method for ubiquitin chains. The method allows us to analyze linkage-type selectivity of ubiquitin decoder proteins at endogenous experimental settings. We recently identified the main pathway targeting K48-linked ubiquitylated substrates for proteasomal degradation. We also identified more complexed ubiquitin chains branched at K48 and K63, which act as a unique coding signal to enhance NF-κB signaling. We are further analyzing the decoder proteins throughout the ubiquitin-mediated pathways to reveal the ubiquitin network.

Fumiaki Ohtake

Hikaru Tsuchiya



Members

Keiji Tanaka	Ai Kaiho
Yasushi Saeki	Naoko Arai
Fumiaki Ohtake	Yuko Okamoto
Hikaru Tsuchiya	Arisa Kawano
Sayaka Yasuda	Marcel Diallo

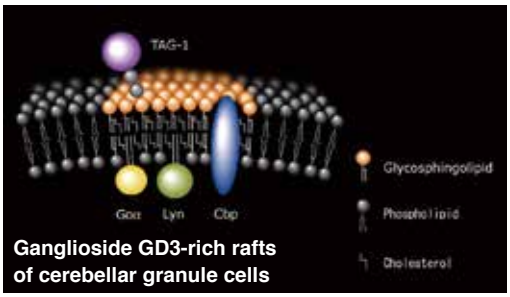
Protein Metabolism

Unit Leader **Kohji Kasahara** Biomembrane Unit

Physiological Functions of Lipid Rafts / Glycosphingolipid Microdomains in Transmembrane Signaling

Lipid rafts are dynamic assemblies of glycosphingolipids, sphingomyelin, cholesterol, and proteins that can be stabilized into microdomains involved in the regulation of a number of cellular processes. We have been investigating the function of lipid rafts by studying the interactions of glycosphingolipids in the nervous system and in blood platelets. We have found that anti-ganglioside GD3 antibodies co-precipitate the GPI-anchored neural cell adhesion molecule TAG-1, src-family kinase Lyn, its substrate Cbp, and the trimeric G protein Goα in cerebellar granule cells, suggesting that these proteins are all found in lipid rafts.

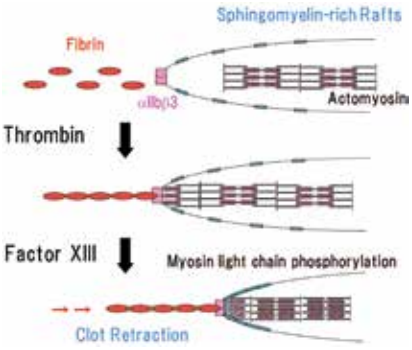
TAG-1 plays roles in axonal guidance and cellular migration, suggesting that it required for transmembrane signal transduction. However, TAG-1 is a GPI-anchored protein and GPI anchors do not directly contact the cytoplasm. We found that TAG-1 transduces signals via interactions with Lyn/Cbp in ganglioside GD3-rich rafts of cerebellar granule cells. The chemokine SDF-1α triggers the chemoattraction of cerebellar granule cells during cerebellar development. We demonstrated that SDF-1α stimulates GTPγS binding to Goα, and causes Goα translocation to lipid rafts, leading to growth cone collapse in cerebellar granule cells.



Ganglioside GD3-rich rafts of cerebellar granule cells

"We found that glycosphingolipids function as platforms in transmembrane signaling for the attachment of various signaling molecules to neurons and platelets."

Fibrin associates with lipid rafts on platelets and raft integrity is required for clot retraction. We propose that clot retraction is mediated by factor XIII-dependent fibrin-integrin αIIbβ3-myosin axis in sphingomyelin-rich membrane rafts.



Sphingomyelin-rich rafts of platelets

Members: Ikuo Kawashima, Kiyoshi Ogura, Tetsuya Hirabayashi
Keisuke Komatsuya, Kei Kaneko

Kasahara K, Kaneda M, Miki T, Iida K, Sekino-Suzuki N, Kawashima I, Suzuki H, Shimonaka M, Arai M, Ohno-Iwashita Y, Kojima S, Abe M, Kobayashi T, Okazaki T, Souri M, Ichinose A, and Yamamoto N. (2013) "Clot retraction is mediated by factor XIII-dependent fibrin-αIIbβ3-myosin axis in platelet sphingomyelin-rich membrane rafts." ***Blood*** 122, 3340-3348.

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Biomembrane